RESEARCH PAPER | Biology | Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

Effect of Furadan on Reduced Glutathione, Lipid Peroxidation and Ascorbic Acid Content of Skin of Psammophilus Blanfordanus

KEYWORDS: Psammophilus blanfordanus, furadan, skin, ascorbic acid, lipid peroxidation, reduced glutathione

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ABSTRACT *Psammophilus blanfordanus were divided into four groups as A, B, C and D. Eeach group comprising of five animals. Animals of group A (control) were given orally 3 µl of acetone/g body weight. The experimental animals of group B, C and D were administered orally with 3 µl of furadan (0.005g of furadan dissolved in 1ml of acetone)/g body weight. The animals were sacrificed after different time intervals such as 0h (group A), 24h (group B), 48h (group C) and 72h (group D). The protein, reduced glutathione (GSH), lipid peroxidation (LPX) and ascorbic acid (ASA) content of skin of the animal were measured and compared at different time intervals.*

INTRODUCTION

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranol methyl carbamate), which is an organocarbamate pesticide and commonly known as furadan, has broad spectrum of action and short half life in the environment. It is therefore widely used as an insecticide, nematicide and acaricide to protect the agricultural and industrial products (Gupta, 1994; Osten *et al.*, 2005; Gera *et al.*, 2011). The application of carbofuran is preferred over the organophosphates (OPs) and organochlorines (OCls) due to its lower toxicity in comparison to OPs and OCls (Agrawal and Sharma, 2010). Lizards and other reptiles have been reported to be more sensitive to the effects of persistent insecticides than are birds and mammals. This apparent sensitivity may result from their low metabolic rate and resultant inability to quickly detoxify contaminants (Hall 1980).

This study was designed to see the toxic effects of furadan on skin of *Psammophilus blanfordanus* by measuring GSH, LPX, Ascorbic acid content of skin at different time intervals of (control) 0h and (experimental) 24h, 48h and 72h .

MATERIALS AND METHODS

Psammophilus (n=20) were caught locally from Baripada, Mayurbhanj, Odisha from the month of January to August. The animals were divided into four groups as A, B, C and D. Eeach group comprising of five animals. Five animals of each group were kept in four different terrarium. They are acclimatized for 7 days in laboratory condition before the experiment. Animals of group A (control) were given orally 3 µl of acetone/g body weight. The experimental animals of group B, C and D were administered orally with 3 µl of furadan (0.005gm of furadan dissolved in 1ml of acetone)/g body weight. The animal after administered orally with acetone or furadan were separated into labeled, perforated plastic bottle. The animals of control group (0hr) were sacrificed immediately, whereas the animals of experimental group B, C and D were sacrificed after 24hr, 48hr and 72hr of treatment. Immediately skin from lower abdomen was dissected out and kept at 0°C. The tissue homogenate was prepared with phosphate buffer (pH 7.4) and then centrifuged at 4000 rpm for 10 minutes in a cold centrifuge machine.

Protein content

Protein estimation of samples was made according to the method of Lowry *et al*. (1961). The data were expressed in mg/g tissue.

Lipid Peroxidation

Lipid peroxidation of the sample was estimated as thiobarbituric acid reacting substance (TBARS) by thiobarbituric acid (TBA) according to the method of Ohkawa *et. al.* (1979). The data were expressed as nmoles of TBARS/mg protein.

Reduced glutathione (GSH)

Glutathione content (GSH) was estimated by the method of Ellman (1959) and the amount of glutathione is expressed as mg/ g tissue.

Ascorbic Acid (ASA)

Ascorbic acid of the sample were estimated by Jagota and Dani (1982) method.

All the solution were prepared by using Millipore distilled water. The above experiments were repeated for 5 times.

RESULTS AND DISCUSSION

Protein content (mg/g tissue) in skin tissue of *Psammophilus blanfordanus* treated with furadan (0.005mg/ml of acetone) were 17.166±0.952 mg/g tissue at 0 hr (control), 14.456±0.87 mg/g tissue at 24 hr, 22.960±1.11 mg/g tissue at 48 hr and 19.984±1.663 mg/g tissue at 72 hr. The protein content (mg/g tissue) of *Psammophilus blanfordanus* exposed to furadan was highest at 48hr ,then decreased at 72 hr but higher than control . The protein content was lowest at 24 hr in comparison to control (Table 1 and Fig 1).

The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is significant with protein content in muscles tissue of *Psammophilus blanfordanus*. [Time interval – protein content (0.572; $P \leq 0.05$)]. One way ANOVA showed that incubation period has significant effect on protein content of liver tissue. Post-hoc analysis revealed

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that only 48 hr is highly significant [F(3,8)=28.368, P< 0.001)]. All the combinations are different from each other (P< 0.001, LSD).

Table-1: Comparison of protein content (mg/g tissue), GSH content (µmol/g tissue), LPX content (nmolTBARS/ mg protein), Ascorbic acid content (mg/g tissue) in skin of Psammophilus blanfordanus treated with furadan. Value is expressed in mean ± S.D.

Time intervals	Protein	GSH	LPX	Ascorbic acid
0 hr	17.166±0.95	0.063±0.005	22.224±1.103	14.89±1.17
24hr	14.456±0.87	0.089±0.0178	20.860±2.1	10.518±1.098
48hr	22.960±1.11	0.031±0.0011	8.686±0.635	15.965±0.88
72hr	19.984±1.63	0.031±0.002	8.486±0.504	13.148±1.034

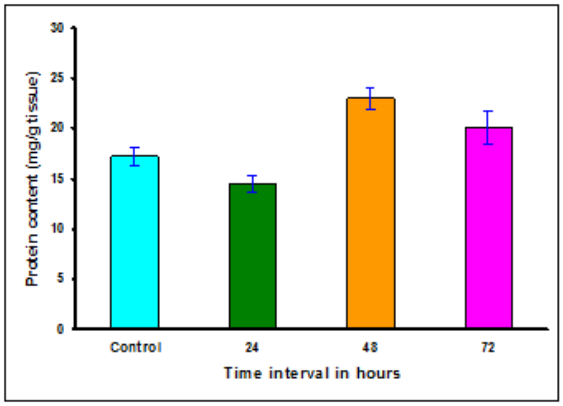

Fig 1. Comparison of protein content in skin
(mg/g tissue) of Psammophilus blanfordanus treated with furadan.

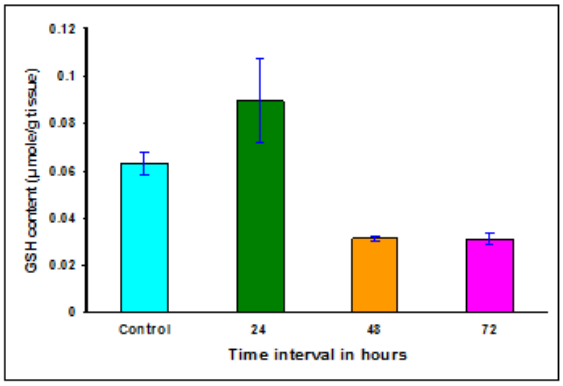

Fig 2. Comparison of GSH content in skin
(mg/g tissue) of Psammophilus blanfordanus treated with furadan.

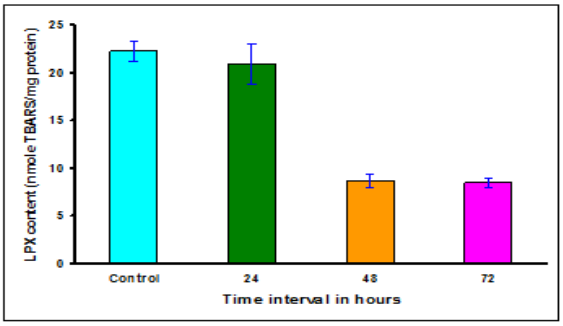

Fig 3. Comparison of LPX content in skin
(mg/g tissue) of Psammophilus blanfordanus treated with furadan.

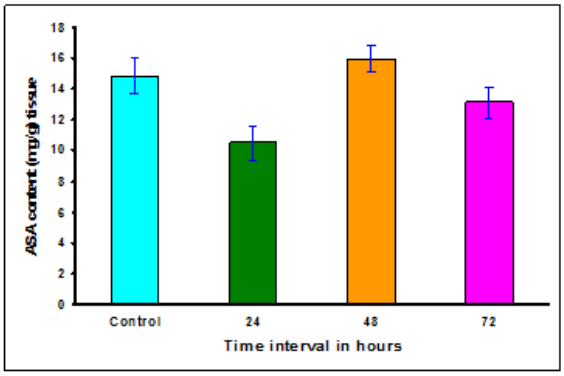

Fig 4. Comparison of ASA content in skin
(mg/g tissue) of Psammophilus blanfordanus treated with furadan.

GSH content (µmol/g tissue) in skin tissue of Psammophilus blanfordanus treated with furadan (0.005mg/ml of acetone) were 0.063033±0.005 µmol/g tissue at 0 hr (control), 0.0892±0.0178 µmol/g tissue at 24 hr, 0.0312±0.0011 µmol/g tissue at 48 hr and 0.031±0.002 µmol/g tissue at 72 hr. The GSH content (µmol/g tissue) was highest at 24hrs and almost equal in 48hr and 72 hr but lower than control groups. (Table 1 and Fig 2)

The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is significant with GSH content in muscles tissue of *Psammophilus blanfordanus*. [Time interval – GSH content (-0.676; P ≤ 0.05)] . One way ANOVA showed that incubation period has significant effect on GSH content of liver tissue. Post-hoc analysis revealed that 24 hr is highly significant [F(3,8)=27.303, P< 0.001)]. All the combinations are different from each other (P< 0.001, LSD).

LPX content (nmol TBARS/mg protein) in liver tissue of *Psammophilus blanfordanus* treated with furadan (0.005mg/ml of acetone) were 22.224±1.103 nmol TBARS/ mg protein at 0hr (control), 20.86±2.1 nmol TBARS/mg protein tissue at 24 hr, 8.686±0.635 nmol TBARS/mg protein tissue at 48 hr and 8.486±0.504 nmol TBARS/mg protein tissue at 72 hr. It was estimated that the concentration of LPX was found highest in 0 hr and decreases from 24 hr to 48hr to 72 hrs in skin tissue of *Psammophilus blanfordanus* (Table 1 and Fig 3).

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The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is significant with LPX content in skin tissue of *Psammophilus blanfordanus*. [Time interval – LPX content (-0.908; $P \leq 0.05$)]. One way ANOVA showed that incubation period has significant effect on LPX content of skin tissue. Post-hoc analysis revealed that only 48 hr is significant [$F(3,8)=107.455$, $P< 0.001$)]. All the combinations are different from each other. ($P< 0.001$, LSD).

Ascorbic acid content (mg/g tissue) in skin tissue of *Psammophilus blanfordanus* treated with furadan (0.005mg/ml of acetone) were14.89±1.17 mg/g at 0 hr (control), 10.518±1.098 mg/g tissue at 24 hr, 15.965±0.88 mg/g tissue at 48 hr and 13.148±1.034 mg/g tissue at 72 hr. It was estimated that the concentration of ascorbic acid was found highest in 48 hr and lowest in 24 hr. The ascorbic acid content at 0 hr was more than 72 hr in skin tissue of *Psammophilus blanfordanus.* (Table 1 and Fig4)

The correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation coefficients (r^2) among the analytical variables showed that the time interval is significant with ascorbic acid content in skin tissue of *Psammophilus blanfordanus*. [Time interval – Ascorbic acid content (0.008; $P \leq 0.05$)]. To test whether the mean analytical variable scores of the four variables differed significantly from each other. One way ANOVA showed that incubation period has significant effect on ascorbic acid content of skin tissue. Post-hoc analysis revealed that only 48 hr is significant [$F(3,8)=15.561$, $P<0.001$)]. All the combinations are different from each other ($P< 0.005$, LSD).

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